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Sequence divergence in a specific region of islet amyloid polypeptide (IAPP) explains differences in islet amyloid formation between species

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Amyloid deposits in the islets of Langerhans occur in association with type 2 diabetes mellitus (DM) in humans and cats and consist of a 37-amino-acid polypeptide known as islet amyloid polypeptide (IAPP). In order to find an explanation for the situation that islet amyloid (IA) does not develop in common rodent species, we have deduced the amino acid sequence of the IAPP molecule in mouse, rat and hamster. We find that a specific region of the molecule diverges to a high degree. Synthetic peptides corresponding to this region of human and hamster IAPP were compared for their ability to form amyloid fibrils in vitro. Whereas the human peptide readily formed fibrils with amyloid character, the hamster peptide completely lacked this property. We suggest this to be a likely explanation for the differences in IA formation between humans and rodents and discuss our findings in relation to the type 2 DM syndrome.

Islet amyloid polypeptide; Diabetes mellitus; Amino acid sequence; Sequence comparison

1. INTRODUCTION

Amyloid deposited in the islets of Langerhans is the most characteristic morphologic islet abnormality in type 2 diabetes mellitus (DM) in humans [1–3] and cats [4,5]. This form of amyloid is composed predominantly of a polymerized 37-amino-acid polypeptide called islet amyloid polypeptide (IAPP) (also identified as diabetes-associated peptide, DAP, or amylin), which is homologous with neuropeptides of the calcitonin-gene related peptide (CGRP) family [6–9]. cDNA cloning of human IAPP indicated that this peptide is a normal processing product of an 89-amino-acid protein precursor [10,11]. The amyloidogenic properties of human IAPP appear to reside in a region spanning amino acid residues 20–29 of the mature molecule in that a synthetic peptide representing this region of IAPP spontaneously aggregates as amyloid fibrils in vitro [11,12].

Age-associated spontaneous-onset type 2 DM occurs only in species that develop islet amyloid (IA). The reason for this difference is not known but we recently performed immunological crossreactivity studies that indicated structural differences in the 20–29 region of IAPP between species that do or do not develop IA [11]. We have now determined the primary structure of IAPP in three rodent species that do not develop islet amyloid and have found a significant amino acid divergence in the 20–29 region compared with human IAPP. A synthetic peptide corresponding to this region of hamster IAPP had quite different chemical properties compared to the corresponding human peptide and did not form amyloid fibrils in vitro. Specific differences in amino acid sequence of IAPP thus appear to be a major cause

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of the variation in islet amyloid formation between mammalian species and may also be relevant for the differences in the development of type 2 DM.

2. MATERIALS AND METHODS

2.1. *Polymerase chain reactions*

Total cellular RNA was prepared [13] from the hamster and rat insulinoma cell lines HIT T15 [14] and RINm4F [15] as well as from mouse pancreas, poly(A) selected on oligo(dT)-cellulose (Pharmacia, Uppsala) and converted to single-stranded cDNA using oligo(dT) primers as described [16]. Approx. 50 ng cDNA was used as template for the enzymatic amplification reaction in which a thermostable *Thermus aquaticus* (Taq) DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) and DNA thermal cycler from the same company were used. The first two cycles had the profile 94°C, 1 min; 37°C, 3 min; 72°C, 15 s and the last 28 cycles had the profile 94°C, 1 min; 55°C, 2 min; 72°C, 15 s with an extra 5 s at 72°C added in each cycle. The following two oligonucleotide primers were employed in the reactions: 5'GCAAGCTTAGTCATCAGGTGGAAAAGCG and 5'CGGAATTCTCTACTGCATTCCTCTTGC. The amplification products were analysed on agarose Tris-borate buffer gels, cleaved with *Eco*RI and *Hin*dIII and subcloned into M13 derivatives for sequence analysis [17]. Both strands were sequenced on several clones to minimize the risk for PCR and sequencing artefacts.

2.2. *Synthetic peptides*

Two peptides corresponding to human and hamster IAPP residues 20–29 were synthesized by automated solid-phase synthesis on an Applied Biosystems model 430A peptide synthesizer (Applied Biosystems, Foster City, CA). The peptides were purified by reversed-phase HPLC on a Nucleosil C18 column followed by mass spectrometry analysis [18]. The human $IAPP_{20-29}$ was solubilized (5 and 20 mg/ml) in 10% NH_4OH and to the clear solution 0.1 M Tris-HCl was added slowly until a gel was formed. In contrast to human $IAPP_{20-29}$, hamster $IAPP_{20-29}$ (5 and 20 mg/ml) easily went into solution in distilled water. Adjustment of the pH to acidic (pH 2) or alkaline (pH 9) values did not result in any visible alteration in the solution. Aliquots of the human and hamster $IAPP_{20-29}$ solutions were dried on glass slides and stained with Congo red. The human but not the hamster material had affinity for the dye and showed a bright green birefringence after this staining. Small droplets of the human and hamster solutions were also applied to formvar coated copper grids, negatively contrasted with 1% uranyl acetate and studied in a Jeol 2000 EX electron microscope at 120 kV. The human material exhibited many slightly wavy fine fibrils of about 40 nm width. Often two filaments were attached side by side resembling amyloid fibrils. In contrast, in the hamster material, only amorphous substance occurred and no fibrils were seen.

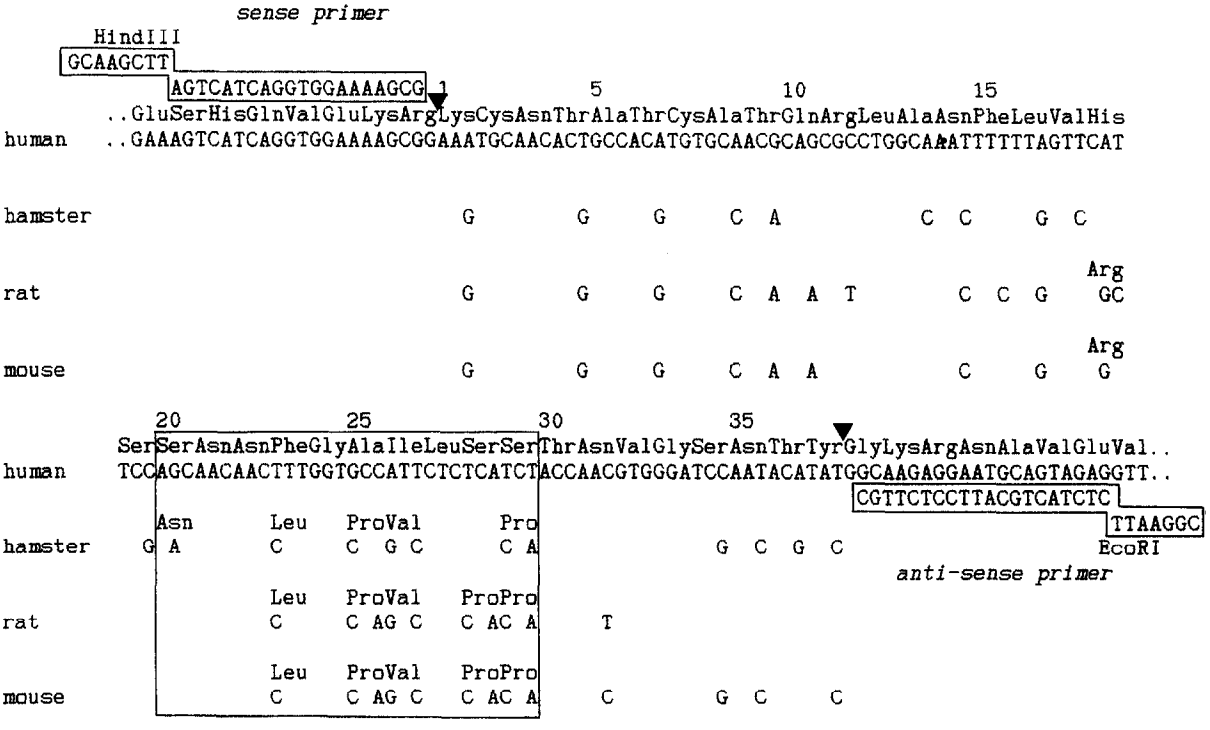

Fig.1. Comparisons between nucleotide and deduced amino acid sequences of human, hamster, rat and mouse IAPP. (▼) N- and C-terminals of mature IAPP. The oligonucleotide primers used for PCR are shown. The 20–29 region of mature IAPP is boxed. For hamster, rat and mouse, only diverging nucleotides and amino acid residues in comparison with the human are shown.

3. RESULTS

Polymerase chain reaction methodology was employed to amplify IAPP sequences in vitro from cDNA prepared using mRNA from the hamster insulinoma cell line HIT T15, the rat insulinoma cell line RINmF4 and mouse pancreas. IAPP mRNA occurs at very low abundance in all these sources. In fact, we have been able to detect a signal on RNA blots only with the HIT T15 cell line [11]. Oligonucleotide primers were designed corresponding to the DNA sequences immediately surrounding the mature human 37-amino-acid IAPP peptide. Their respective 3′ ends thus fall within the proteolytic cleavage sites surrounding IAPP which are likely to be conserved between different species. Amplified DNA fragments were subcloned into M13 derivatives and sequenced. Fig.1 shows the resulting hamster, rat and mouse IAPP DNA and protein sequences in comparison with human IAPP. Whereas the amino-terminal 17 residues and the carboxy-terminal 8 residues are identical in the four species, substantial divergence is seen between residues 18 and 29. Notably, all five amino acid differences in hamster vs human and five out of six diverging positions in rat and mouse fall within residues 20–29 of the IAPP molecule. A synthetic peptide corresponding to this region in human IAPP has previously been shown to form fibrils having the characteristics of amyloid as judged by ultrastructural morphology and exhibition of green birefringence following Congo red staining. A synthetic peptide corresponding to the same region in hamster completely lacked these properties, however (fig.2). This result is in agreement with the lack of islet amyloid formation in hamsters and suggests that amino acid sequence differences in the 20–29 region of IAPP are

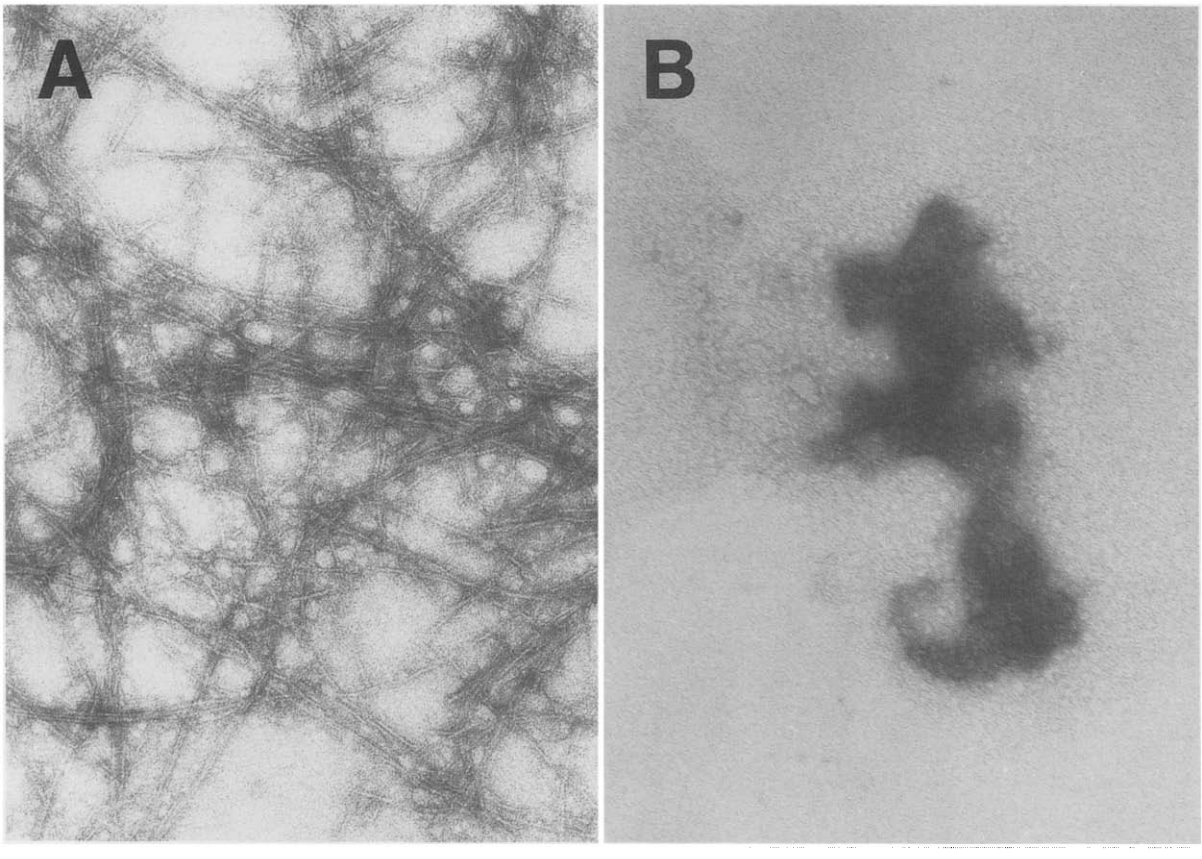

Fig.2. Electron micrographs showing synthetic fibrils formed by human $IAPP_{20-29}$ (A). Hamster $IAPP_{20-29}$ (B) did not give rise to any amyloid-like fibrils. × 120000.

responsible for the observed species-specific pattern of islet amyloid formation.

4. DISCUSSION

The potential role of islet amyloid deposition in the pathogenesis of type 2 DM has been largely ignored in spite of the fact that its occurrence in association with the disease was first reported almost 90 years ago [20]. Interest in this phenomenon has been revived lately through the identification of the major amyloid constituent as an islet β-cell product related to the CGRPs [6–9]. Subsequently, the demonstration of a biological function of IAPP as an inhibitor of insulin-stimulated glucose uptake in skeletal muscle cells [21] has attracted considerable interest in relation to one of the obligatory signs of type 2 DM, peripheral insulin resistance (see [22]). The type 2 DM syndrome also includes a variable defect in insulin secretion from the β-cells [23,24] and we recently pointed out that the role of IAPP in type 2 DM might be dual, i.e. disturbance of islet β-cell function by its local deposition as amyloid (which causes destruction of the β-cell membrane and which might act as a diffusion barrier) and inhibition of the peripheral insulin function through increased circulating levels of IAPP [25]. The former argument is also supported by a correlation between immunological cross-reactivity in the 20–29 region of IAPP and the observed tendency to develop islet amyloid in association with spontaneous maturity-onset DM in different species [11]. The present data show that the amino acid sequence divergence in IAPP occurs almost exclusively in what has previously been identified as the amyloidogenic region of human IAPP. That this region of hamster IAPP does not possess in vitro amyloidogenic properties highlights the questions regarding a possible role of islet amyloid deposition in the development of type 2 DM.

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